

APPLICATION

for

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on

GENETIC MARKER TEST FOR LUPUS

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## GENETIC MARKER TEST FOR LUPUS

This application is a continuation-in-part of pending U.S. Serial No. 09/280,181, filed on March 29, 1999.

### BACKGROUND OF THE INVENTION

Throughout this application various publications are referenced within parentheses. The disclosures of these publications in their entireties are hereby incorporated by reference in this application in order to more fully describe the state of the art to which this invention pertains.

#### 1. THE FIELD OF THE INVENTION

This invention relates to the medical arts. In particular, it relates to a method of detecting a genetic predisposition to systemic lupus erythematosus.

#### 2. DISCUSSION OF THE RELATED ART

Systemic lupus erythematosus (SLE), commonly known as lupus, is an autoimmune rheumatic disease characterized by deposition in tissues of autoantibodies and immune complexes leading to tissue injury (B.L. Kotzin, *Systemic lupus erythematosus*, Cell 85:303-06 [1996]). In contrast to autoimmune diseases such as multiple sclerosis and type 1 diabetes mellitus, SLE potentially involves multiple organ systems directly, and its clinical manifestations are diverse and variable. (Reviewed by B.L. Kotzin and J.R. O'Dell, *Systemic lupus erythematosus*, In: *Samler's Immunologic Diseases*, 5th ed., M.M. Frank *et al.*, eds., Little Brown & Co., Boston, pp. 667-97 [1995]).

For example, some patients may demonstrate primarily skin rash and joint pain, show spontaneous remissions, and require little medication. At the other end of the spectrum are patients who demonstrate severe and progressive kidney involvement that requires therapy with high doses of steroids and cytotoxic drugs such as cyclophosphamide. (B.L. Kotzin [1996]). The serological hallmark of SLE, and the primary diagnostic test available until now, is elevated serum levels of

IgG antibodies to constituents of the cell nucleus, such as double-stranded DNA (dsDNA), single-stranded DNA (ss-DNA), and chromatin. Among these autoantibodies, IgG anti-dsDNA antibodies play a major role in the development of lupus glomerulonephritis (GN). (B.H. Hahn and B. Tsao, *Antibodies to DNA*, In: *Dubois' Lupus Erythematosus*, 4th ed., D.J. Wallace and B. Hahn, eds., Lea and Febiger, Philadelphia, pp. 195-201 [1993]; Ohnishi *et al.*, *Comparison of pathogenic and nonpathogenic murine antibodies to DNA: Antigen binding and structural characteristics*, Int. Immunol. 6:817-30 [1994]). Glomerulonephritis is a serious condition in which the capillary walls of the kidney's blood purifying glomeruli become thickened by accretions on the epithelial side of glomerular basement membranes. The disease is often chronic and progressive and may lead to eventual renal failure.

Mechanisms by which autoantibodies are induced remain unclear. Chromatin and/or nucleosomes, released by apoptotic cells in SLE, may become autoantigens that induce autoimmune responses, including antibodies to dsDNA. (R.W. Burlingame *et al.*, *The central role of chromatin in autoimmune responses to histones and DNA in systemic lupus erythematosus*, J. Clin. Invest. 94:184-92 [1994]; C. Mohan *et al.*, *Nucleosome: a major immunogen for pathogenic autoantibody-inducing T cells of lupus*, J. Exp. Med. 177:1367-81 [1993]; D.A. Bell *et al.*, *Immunogenic DNA-related factors. Nucleosomes spontaneously released from normal murine lymphoid cells stimulate proliferation and immunoglobulin synthesis of normal mouse lymphocytes*, J. Clin. Invest. 85:1487-96 [1990]).

Cumulative studies suggest that interaction of multiple genes and environmental factors result in susceptibility to SLE, as is true for many multifactorial complex human diseases. (F.C. Arnett, Jr., *The genetics of human lupus*, In: *Dubois' Lupus Erythematosus*, 5th ed., D.J. Wallace and B. Hahn, eds., Williams and Wilkins, Baltimore, pp. 77-117 [1997]; T.J. Vyse and B.L. Kotzin, *Genetic susceptibility to systemic lupus erythematosus*, Ann. Rev. Immunol. 16:261-92 [1998]).

Although SLE can occur at nearly any age, it primarily affects women of childbearing age; the female to male ratio is greatest (>8:1) for patients presenting between ages 15 to 50 years; incidence rates for patients and studies in certain animal models support a role for estrogens enhancing disease development, and androgens protecting against it. (B.L. Kotzin [1996]). Although rare among males, SLE may be linked in males with functional hypoandrogenism and a

higher than normal estradiol/testosterone ratio. (J.F. Sequeira *et al.*, *Systemic lupus erythematosus: sex hormones in male patients*, *Lupus* 2(5):315-17 [1993]).

It appears that underlying genetic factors exert the greatest influence on autoantibody production and on predisposition to SLE, as studies of populations, segregation of disease in families, and twin concordance rates have consistently demonstrated. The prevalence of SLE in the general population is approximately 15-50 in 100,000. (M.D. Hochberg, *The epidemiology of systemic lupus erythematosus*, In: *Dubois' Lupus Erythematosus*, 5th ed., D.J. Wallace and B. Hahn, eds., Williams and Wilkins, Baltimore, pp. 49-65 [1997]). The relatively high incidence (10-16%) of more than one case in a family has suggested a genetic basis for SLE. The concordance rate of SLE in monozygotic twins (24% - 57%) is approximately ten times higher than the rate in dizygotic twins (2-5%). (F.C. Arnett, Jr. [1997]; M.D. Hochberg [1997]). Based on these epidemiological studies, the relative risk for siblings of SLE patients compared to the general population,  $\lambda_s$ , is at least 40-fold. (See, N. Risch, *Assessing the role of HLA-linked and unlinked determinants of disease*, *Am. J. Hum. Genet.* 40:1-14 [1987]).

The genetic basis for SLE in humans is complex, with an unknown but non-Mendelian mode of inheritance. This complexity has impeded the development of a reliable and predictive genetic test for SLE until the present invention.

Many investigators have reported that certain human MHC class II alleles (*HLA-DR* and/or *DQ* but not *DP*) and certain class III genes (*C2*, *C4*, *TNF $\alpha$*  and *HSP70-2* alleles) confer susceptibility to SLE in most ethnic groups studied. (F.C. Arnett, Jr., *The genetics of human lupus*, In: *Dubois' Lupus Erythematosus*, 5th ed., D.J. Wallace and B. Hahn, eds., Williams and Wilkins, Baltimore, pp. 77-117[1997]; T.J. Vyse and B.L. Kotzin, *Genetic susceptibility to systemic lupus erythematosus*, *Ann. Rev. Immunol.* 16:261-92 [1998]). Among the other non-MHC genes that have been associated with SLE, evidence for homozygous deficiency of *C1q* predisposing to SLE is particularly compelling, including the observation that 90% of such individuals have SLE and *C1q* knockout mice display an SLE-like phenotype. (M. Botto *et al.*, *Homozygous C1q deficiency causes glomerulonephritis associated with multiple apoptotic bodies*, *Nat. Genet.* 19:56-59 [1998]; P. Bowness *et al.*, *Hereditary C1q deficiency and systemic lupus erythematosus*, *Quart. J. Med.* 87:455-64 [1994]).

In addition, polymorphisms in many genes encoding molecules with relevant immunological functions have been studied most frequently by the case-control approach, including T-cell receptor  $\alpha$  and  $\beta$  chains, immunoglobulin allotypes, Fc $\gamma$ RIIa, Fc $\gamma$ RIIIa, *IL-6*, *IL-10*, *Bcl-2*, mannose-binding protein (or lectin), as well as deletion of specific variable gene segments of immunoglobulin genes.

(F.C. Arnett, Jr. [1997]; T.J. Vyse and B.L. Kotzin [1998]; J. Wu *et al.*, *A novel polymorphism of Fc $\gamma$ RIIIA, which alters function, associates with SLE phenotype*, J. Invest. Med. 45:200A [1997]; R. Mehrian *et al.*, *Synergistic effect between IL-10 and Bcl-2 genotypes in determining susceptibility to systemic lupus erythematosus*, Arthritis Rheum. 41:596-602 [1998]). Mutations in nucleic acids encoding T cell receptor zeta chain have been linked to SLE in some patients. (K. Tsuzaka *et al.*, *Mutations in T cell receptor zeta chain mRNA of peripheral T cells from systemic lupus erythematosus patients*, J. Autoimmun. 11(5):381-85 [1998]). Some candidate genes may confer risk only to subsets of SLE patients. For example, Fc $\gamma$ RIIA alleles (the gene encoding a 40-kD Fc $\gamma$ R expressed on human mononuclear phagocytes and neutrophils) confer an increased risk for lupus GN in African Americans, but not in Caucasians, or persons of Afro-Caribbean or Chinese origin. (J.E. Salmon *et al.*, *Fc $\gamma$ RIIA alleles are heritable risk factors for lupus nephritis in African-Americans*, J. Clin. Invest. 97:1348-54 [1996]; M. Botto *et al.*, *Fc $\gamma$ RIIA polymorphism in systemic lupus erythematosus [SLE]*, Clin. Exp. Immunol. 104:264-68 [1996]).

Elements that complicate the study of disease-causing genes in genetically complex diseases, such as human SLE, include ethnic diversity, clinical heterogeneity (and presumably genetic heterogeneity), reduced penetrance (genetic expressivity), and the effect of environment (E.S. Lander and N.J. Schork, *Genetic dissection of complex traits*, Science 265:2037-48 [1994]). In contrast, murine models of spontaneous lupus in inbred strains are less complex, and consequently murine models of disease susceptibility provide a more accessible route for investigating genetically-linked disease in humans. Recent success in mapping a susceptibility locus for multiple sclerosis in the 5p14-p12 region, which is syntenic to the murine locus Ea2, further supports the utility of this mouse-to-human approach. (S. Kuokkanen *et al.*, *A putative vulnerability locus to multiple sclerosis maps to 5p14-p12 in a region syntenic to the murine locus Eae2*, Nature Genet. 13:477-80 [1996]).

Genetic studies of murine SLE have identified susceptibility loci in several inbred strains

which spontaneously develop SLE GN. (Reviewed in A.N. Theofilopoulos, *The basis of autoimmunity: Part II. Genetic predisposition*, Immunology Today 15:150-58 [1995]). These studies have included genome-wide searches for evidence of linkage using backcrosses or F<sub>2</sub> intercrosses of lupus mice such as MRL/LPR, NZB/NZW and NZM/Aeg2410 (M.L. Watson *et al.*, *Genetic analysis of MRL-lpr mice: Relationship of the Fas apoptosis gene to disease manifestations and renal disease-modifying loci*, J. Exp. Med. 176:1645-56 [1992]; D.H. Kono *et al.*, *Lupus susceptibility loci in New Zealand mice*, Proc. Natl. Acad. Sci. USA 91:10168-72 [1994]; Drake *et al.*, *Analysis of the New Zealand Black contribution to lupus-like renal disease: multiple genes that operate in a threshold manner*, J. Immunol. 154:241-47 [1995]; Drake *et al.*, *Genetic analysis of the NZB contribution to lupus-like autoimmune disease in [NZB x NZW]F<sub>1</sub> mice*, Proc. Natl. Acad. Sci. USA 91:4062-66 [1994]; S. Hirose *et al.*, *Mapping of a gene for hypergammaglobulinemia to the distal region chromosome 4 in NZB mice and its contribution to systemic lupus erythematosus in [NZB x NZW]F<sub>1</sub> mice*, Internat. Immunol. 12:1857-64 [1994]; L. Morel *et al.*, *Polygenic control of susceptibility to murine SLE*, Immunity 1:219-229 [1994]).

Four genomic intervals strongly linked to GN in mice derived from different parental strains have been identified in multiple studies at loci on chromosomes 1, 4, 7, and 17. The distal end of mouse chromosome 1 was shown to predispose to specific manifestations of SLE, including GN, IgG anti-chromatin antibodies, and splenomegaly. (D.H. Kono *et al.* [1994]; Drake *et al.* [1995]; L. Morel *et al.* [1994]).

There is evidence for a corresponding genetic linkage in human SLE. Using the identified murine susceptibility loci (the overlapping *SLE/Nba2/Lbw7*) as a guide, Tsao *et al.* examined seven markers located on a syntenic human chromosomal 1q31-q42 region, corresponding to the telomeric end of mouse chromosome 1, the latter being the region where contributions to specific manifestations of murine lupus, including glomerulonephritis and IgG anti-chromatin, have been mapped. (B. Tsao *et al.*, *Evidence for linkage of a candidate chromosome 1 region to human systemic lupus erythematosus*, J. Clin. Invest. 99:725-731 [1997]). The seven markers were examined in 52 affected human sibpairs from Caucasian, Asian and African-American families. Five markers located in a 15 cM region of human chromosome 1q41-q42 showed evidence for linkage excessive by the allele sharing method (B. Tsao *et al.* [1997]; B. Tsao *et al.*, *The genetic basis of*

systemic lupus erythematosus, Proc. Assoc. Am. Physicians 110(2):113-17 [Review Mar.-Apr. 1998]]. Subsequently, an independent sample of 105 SLE-affected sibpairs also supported linkage of the 1q41-q42 region with SLE. (P.M. Gaffney *et al.*, *A genome-wide search for susceptibility genes in human systemic lupus erythematosus sib-pair families*, Proc. Natl. Acad. Sci. USA [in press]).

The distance (>60 cM) between the 1q41-q42 region and the FcγRIIa gene (1q23) make it unlikely that this gene could account for the observed linkage between the 1q41-q42 region and SLE. A polymorphism for low expression of *CR1* (complement receptor one, previously C3b/C4b receptor) has been suggested to be a risk factor for SLE (P. Cornillet *et al.*, *Increased frequency of the long (S) allotype of CR1 (the C3b/C4b receptor, CD35) in patients with systemic lupus erythematosus*, Clin. Exp. Immunol. 89:22-25 [1992]), although a later study suggests that low *CR1* expression is acquired. (A. Kumar *et al.*, *HindIII genomic polymorphism of the C3b receptor (CR1 in patients with SLE: low erythrocyte CR1 expression in acquired phenomenon*, Immunol. Cell Biol. 73:457-62 [1995]). However, the *CR1* gene maps to chromosome 1q32, and linkage disequilibrium at the 1q41-42 region, but not at the 1q31-32 region, make *CR1* an unlikely candidate as a susceptibility gene for SLE. (B. Tsao *et al.* [1997]).

Within the human chromosomal 1q41-q42 region, there are three candidate genes for linkage with an SLE phenotype. One of these, *HLX1*, is expressed in hematopoietic progenitors and activated lymphocytes and encodes a homeo box protein which may regulate the development of CD4<sup>+</sup> T-cells. (Y. Deguchi *et al.*, *A human homeobox gene, HB24, inhibits development of CD4<sup>+</sup> T cells and impairs thymic involution in transgenic mice*, J. Biol. Chem. 268:3646-53 [1993]). Another, *TGFB2* (transforming growth factor beta-2) can suppress Il-2 dependent T-cell growth (R. Demartin *et al.*, *Complementary DNA for human glioblastoma-derived T cell suppressor factor, a novel member of the transforming growth factor-beta gene family*, EMBO J. 6:3673-77 [1987]), and administration of a TGFβCDNA expression vector to MRL/lpr mice decreases serum IgG anti-chromatin and delays the onset of SLE. (E. Raz *et al.*, *Modulation of disease activity in murine systemic lupus erythematosus by cytokine gene delivery*, Lupus 4:286). However, *TGFB2* is not expressed in hematopoietic cells, and such expression is believed to be an essential link in the etiology of SLE.

The third candidate gene, *PARP*, sometimes referred to as *ADPRT* (ADP-ribosyltransferase gene), is induced by DNA damage and plays a role in cellular repair (P. Loetscher *et al.*, *Poly(ADP-ribose) may signal changing metabolic conditions to the chromatin of mammalian cells*, Proc. Natl. Acad. Sci. USA 84:1286-89 [1987]). Haug *et al.* reported that SLE patients and their family members have decreased poly(ADP-ribose) metabolism. (B.L. Haug *et al.*, *Altered poly(ADP-ribose) metabolism in family members of patients with systemic lupus erythematosus*, J. Rheum. 21:851-56 [1994]), which is evidence that *PARP* is linked to SLE.

The *PARP* gene encodes poly(ADP-ribosyl)transferase, also known as poly(ADP-ribosyl)polymerase, which is a zinc-finger DNA-binding protein that repairs DNA damage and is specifically cleaved during apoptosis. (B. Tsao *et al.*, *ADPRT alleles from the chromosome 1q41-q42 linked region are associated with SLE*, Arthritis & Rheumatism 41(9)(Suppl.):283 [Abstract, Sept. 1998]). In their abstract, Tsao *et al.* reported that none of the tested alleles of *HLX1* showed evidence for association with SLE phenotypes in a family-based linkage test applied to 124 families. However, a polymorphism within the *PARP* region was associated with SLE phenotype. This polymorphism was reported to be a dinucleotide repeat in the *PARP* promoter region.

There has been a need for a rapid and accurate genetic test for SLE. Previously available diagnostic tests for SLE are limited in their diagnostic effectiveness. Rheumatoid factors (RF), antinuclear antibodies (ANA), and erythrocyte sedimentation rate (ESR) are among the most frequently used tests for the diagnosis and investigation of connective tissue disease, including SLE. However, positive predictive values for these diagnostic tests are reported to be low as to a diagnosis of SLE, albeit they may be more predictive for a subject having other clinical SLE symptoms from among the 11 revised SLE criteria of the American College of Rheumatology (ACR), such as a typical malar or discoid rash, photosensitivity, oral ulcers, arthritis, serositis, or disorders of blood, kidney or nervous system. (E.M Tan *et al.*, *The 1982 revised criteria for the classification of systemic lupus erythematosus [SLE]*, Arthritis Rheum. 25:1271-77 [1982]; M.E. Suarez-Almazor *et al.*, *Utilization and predictive value of laboratory tests in patients referred to rheumatologists by primary care physicians*, J. Rheumatol. 25(10):1980-85 [1998]; C.A. Slater *et al.*, *Antinuclear antibody testing. A study of clinical utility*, Arch. Intern. Med. 156(13):1421-25[1996]). The diagnostic utility of ELISA assay for the presence of anti-extractable nuclear



antigen (anti-ENA) antibodies is limited to those patients that also test positive for both ANA and anti-dsDNA antibodies. (J. Sanchez-Guerrero *et al.*, *Utility of antiSm, anti-RNP, anti-Ro/SS-A, and anti-La/SS-B [extractable nuclear antigens] detected by enzyme-linked immunosorbent assay for the diagnosis of systemic lupus erythematosus*, Arthritis Rheum. 39(6):1055-61 [1996]).

Unfortunately, a patient's presentation of clinical symptoms in SLE may be vague or idiopathic, easily mistaken for another disorder. For example, uncommon clinical manifestations associated with underlying SLE may include, acute pancreatitis, pure red cell aplasia, cystitis of the urinary tract, antiphospholipid antibody syndrome, or neurological complications of normal pressure hydrocephalus. (K.P. Leong and M.L. Boey, *Systemic lupus erythematosus [SLE] presenting as acute pancreatitis-- a case report*, Singapore Med. J. 37(3):323-24 [1996]; M.H. Tsai *et al.*, *Systemic lupus erythematosus with pure red cell aplasia: a case report*, Chung Hua I Hsueh Tsa Chih [Taipei] 52(4):265-68 [1993]; Y. Nakauchi *et al.*, *Systemic lupus erythematosus relapse with lupus cystitis*, Clin. Exp. Rheumatol. 13(5):645-48 [1995]; P.R. Mortifee *et al.*, *Communicating hydrocephalus in systemic lupus erythematosus with antiphospholipid antibody syndrome*, J. Rheumatol. 19(8):1299-1302 [1992]; H.Y. You *et al.*, *Normal pressure hydrocephalus in a patient with systemic lupus erythematosus: a case report*, Chung Hua I Hsueh Tsa Chih [Taipei] 61(9):551-55 [1998]; M.D. Uhl *et al.*, *Normal pressure hydrocephalus in a patient with systemic lupus erythematosus*, J. Rheumatol. 17(2):1689-91 [1990]).

Consequently, a genetic testing method for SLE has been needed that can be used in conjunction with other diagnostic tests for SLE. This, and other advantages described herein, the present invention provides.

## SUMMARY OF THE INVENTION

The present invention relates to a genetic testing method for diagnosing the autoimmune disease systemic lupus erythematosus in a human subject. In particular, the claimed invention is directed to amplifying nucleic acids from a tissue sample of a human subject, analyzing or detecting the amplification products for the presence of a variant allele in *PARP*, the gene which encodes for expression of poly(ADP-ribosyl) transferase. A variant allele of *PARP* is diagnostic of SLE in a human subject having symptoms of SLE or indicates a genetic predisposition for SLE in a subject

who does not present SLE symptoms. The methods of the present invention provide a hitherto unavailable genetic test for susceptibility to this potentially life-threatening disease.

The method of the present invention relates to amplification of the nucleic acids in a human tissue sample using oligonucleotide primers specific for a nucleic acid sequence derived from the genomic sequence of human chromosome 1q41-q42 between microsatellite markers D1S2860 and D1S213. The present invention also relates to a genetic testing method for diagnosing SLE by amplifying nucleic acids having a variant allele in a *PARP* promoter region, for example, the 85 base pair or 97 base pair alleles, which are associated, respectively, with increased or decreased genetic susceptibility to SLE in subjects from diverse ethnic origins.

Another feature of the present invention are oligonucleotide primer sequences used for detecting a genetic susceptibility to SLE, a primer set with which to practice the present method, and a genetic testing kit comprising these primers or the primer set.

The present invention provides a rapid and accurate genetic test for SLE. It can be applied diagnostically in conjunction with other available diagnostic tests in suspected cases of SLE. The methods, primers, and genetic testing kits of the present invention can also be applied so that a person without any of the symptoms of SLE, but who is found to possess a genetic predisposition for SLE, can be treated to minimize his or her exposure to environmental risk factors in order to prevent the development of SLE.

These and other advantages and features of the present invention will be described more fully in a detailed description of the preferred embodiments which follows, and by way of the disclosures of U.S. Serial No. 09/280,181, filed on March 29, 1999, which are incorporated by reference.

#### BRIEF DESCRIPTION OF THE DRAWING

Figure 1 illustrates the results of multipoint linkage analysis of the chromosome 1q31-q42 region using the MAPMAKER/SIBS program. Positions of genetic markers relative to D1S510 are expressed in centiMorgans (cM) as the relative genetic distance shown in the x-axis.

## DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The present invention relates to a genetic testing method for diagnosing systemic lupus erythematosus in a human subject. The present invention provides a hitherto unavailable genetic testing method for susceptibility to this potentially life-threatening disease. In particular, the claimed invention is directed to amplifying nucleic acids from a tissue sample of a human subject, analyzing or detecting the amplification products for the presence of a variant allele in *PARP*, a gene encoding for expression of poly(ADP-ribosyl) transferase. A variant allele of *PARP* is diagnostic of SLE in a subject having clinical SLE symptoms or indicates a genetic predisposition for developing SLE in a subject who does not present SLE symptoms.

A *PARP* gene, for the purposes of the present invention, is a gene that encodes poly(ADP-ribosyl) transferase. Poly(ADP-ribosyl) transferase (E.C. 2.4.2.30) functions in the maintenance of genomic integrity; it is the only enzyme known to synthesize ADP-ribose polymers from nicotinamide adenine dinucleotide (NAD<sup>+</sup>) and is activated in response to DNA strand breaks. (W.M. Shieh *et al.*, *Poly(ADP-ribosyl)polymerase null mouse cells synthesize ADP-ribose polymers*, J. Biol. Chem. 273(46):30069-72 [1998]). Poly(ADP-ribosyl) transferase enzyme has been shown to stimulate DNA polymerase  $\alpha$  by physical association and may form a complex with DNA polymerase  $\alpha$  in vivo. (C.M. Simbulan *et al.*, *Poly(ADP-ribose)polymerase stimulates DNA polymerase alpha by physical association*, J. Biol. Chem. 268(1):93-99 [1993]). Activation of poly(ADP-ribosyl) transferase requires both the DNA-binding capacity of the DNA-binding domain ("zinc fingers") and the ability to maintain a conformation of the DNA-binding domain that can transfer an "activation signal" to the catalytic domain of the enzyme. (C. Trucco *et al.*, *Mutations in the amino-terminal domain of the human poly(ADP-ribose)polymerase that affect its catalytic activity but not its DNA-binding capacity*, FEBS Lett. 399(3):313-16 [1996]).

The important physiologic function of poly(ADP-ribosyl) transferase has been extensively studied by using specific inhibitors (3-aminobenzamide, 3-methoxybenzamide, or antisense RNA) and by studies of knockout mice. (P.A. Jeggo, *DNA repair: PARP-another guardian angel?*, Current Biol. 8:R49-R51 [1998]). Cumulative data have shown that the absence of poly(ADP-ribosyl) transferase activity results in elevated spontaneous genetic rearrangements and hypersensitive responses to DNA damage, implying a substantial role for poly(ADP-ribosyl)

transferase in maintaining genomic stability. Although no gross defects in apoptosis are found in *PARP* knockout mice, splenocytes of these mice display a more rapid apoptotic response to an alkylating agent. Cell lines with disrupted *PARP* expression show insensitivity to apoptotic signals. (C. M. Simbulan-Rosenthal *et al.*, *Transient poly(ADP-ribosyl)ation of nuclear proteins and role of poly(ADP-ribose)polymerase in the early stages of apoptosis*, J. Biol. Chem. 273:13703-12 [1998]). While *PARP* has a regulatory role in induced apoptosis, impaired apoptosis is less detectable in whole animals than in cell lines, probably because of other compensatory routes within the organism.

The present invention is not committed to any particular mechanism by which altered *PARP* expression or various *PARP* alleles may produce symptoms of, or susceptibility to, SLE. However, it is hypothesized that the 85 bp allele of *PARP*, or other variant alleles of *PARP*, confers defective DNA repair and abnormal apoptosis, and thus predisposes to SLE. Further support for this hypothesis of the direct involvement of *PARP* gene product in SLE predisposition includes a lower than normal range of poly(ADP-ribosyl) transferase activity in SLE patients, an intermediate range of activity in unaffected family members of SLE patients, and decreased levels of *PARP* mRNA transcripts in SLE patients. (S.L. Oei *et al.* [1997]; P.A. Jeggo [1998]). The specific examples provided herein demonstrate that *PARP* is a SLE susceptibility gene.

Human *PARP* is located on chromosome 1 at locus 1q41-q42. (H. Herzog *et al.*, *Human nuclear NAD<sup>+</sup> ADP-ribosyltransferase: localization of the gene on chromosome 1q41-q42 and expression of an active human enzyme in Escherichia coli*, Proc. Natl. Acad. Sci. USA 86(10):3514-18 [1989]). More specifically, the *PARP* gene region is flanked by microsatellite markers D1S2860 and D1S213. (See Figure 1)(C. Dib *et al.*, *A comprehensive genetic map of the human genome based on 5,264 microsatellites*, Nature 380(6570):152-54 [1996]). The *PARP* gene is 43 kb in length and is split into 23 exons. (B. Auer *et al.*, *Human nuclear NAD<sup>+</sup> ADP-ribosyltransferase: organization of the gene*, DNA 8(8):575-80 [1989]). Each of the four metal coordinating sites forming the two "zinc fingers" of the poly(ADP-ribosyl) transferase DNA-binding domain is encoded separately; the automodification and NAD<sup>+</sup>-binding domains are encoded by four and twelve exons, respectively. Nucleotide sequences of the entire human *PARP* genomic region are known, including nucleotide sequences defining regulatory and open reading

frame regions. (H. Suzuki *et al.*, *Molecular cloning of cDNA for human poly(ADP-ribose)polymerase and expression of its gene during HL-60 cell differentiation*, Biochem. Biophys. Res Commun. 146(2):403-09 [1987]; B.W. Cherney *et al.*, *cDNA sequence, protein structure, and chromosomal location of the human gene for poly(ADP-ribose)polymerase*, Proc. Natl. Acad. Sci. USA 84(23):8370-74 [1987]; T. Ogura *et al.*, *Characterization of a putative promoter region of the human poly(ADP-ribose)polymerase gene: structural similarity to that of the DNA polymerase beta gene*, Biochem. Biophys. Res. Commun. 167(2):701-10 [1990]; Y. Yokoyama *et al.*, *Human poly(ADP-ribose)polymerase gene. Cloning of the promoter region*, Eur. J. Biochem. 194(2):521-26 [1990]). Several restriction fragment length polymorphisms of the *PARP* gene are also known. (B.W. Cherney *et al.* [1987]).

Variant alleles of the *PARP* gene include mutations or polymorphisms of any class. Many variant alleles of *PARP* are known. These include several single point mutations that cause a loss of poly(ADP-ribosyl) transferase catalytic activity. (C. Trucco *et al.*, [1996]). But for purposes of the present invention, variant alleles of *PARP* are not limited to such single nucleotide polymorphisms (SNPs). Also included among variant alleles of *PARP* are deletion mutations, insertion mutations, inversions, translocations, transitions, tranversions, or repeats. An example, is the dinucleotide CA repeat that occurs in the 85 bp allele of a *PARP* promoter region; this dinucleotide repeat is located about 907 bp upstream from the transcription start site, near the binding site of the transcription factor Ying Yang 1. (S.L. Oei *et al.*, *Interaction of the transcription factor YY1 with human poly(ADP-ribosyl)transferase*, Biochim. Biophys. Res. Comm. 240:108-11 [1997]).

Variant alleles of a *PARP* gene may be of any nucleotide sequence defining an open reading frame, or part thereof, that encodes any subunit of the catalytic or automodification domain, or the DNA-binding domain, or the NAD<sup>+</sup>-binding domain of poly(ADP-ribosyl) transferase, or of any nucleotide sequence, or part thereof, defining a regulatory region of a *PARP* gene. A *PARP* regulatory region may include, but is not limited to, a promoter region, an enhancer, a termination sequence, or any other nucleotide sequence, or part thereof, that regulates the transcription of poly(ADP-ribosyl) transferase protein. Any mutation or polymorphism in a *PARP* gene that results in a loss of *PARP* expression or a decrease in poly(ADP-ribosyl) transferase functional activity

relative to a normal activity range, or which could do so if it were not masked by the presence in vivo of a fully functional *PARP* allele or by another compensatory physiologic mechanism, are contemplated by the present invention.

Any human tissue containing nucleic acids may be sampled and collected for the purpose of practicing the methods of the present invention. A most preferred and convenient tissue for collecting is blood. "Collecting a tissue sample" encompasses in vitro harvest of cultured human cells derived from a subject's tissue or any means of in vivo sampling directly from a subject, for example, by blood draw, spinal tap, tissue smear or tissue biopsy. Tissue samples may be stored before analysis by well known storage means that will preserve a sample's nucleic acids in an analyzable condition, such as quick freezing, or a controlled freezing regime, in the presence of a cryoprotectant, for example, dimethyl sulfoxide (DMSO), glycerol, or propanediol-sucrose. Tissue samples may also be pooled before or after storage for purposes of amplifying them for analysis.

For purposes of the present invention "amplifying nucleic acids" from a tissue sample of a subject "to obtain amplification products" encompasses any conventional means of amassing sufficient nucleic acid material for analysis. This includes, but is not limited to, amplification by conventional polymerase chain reaction (PCR) methods. Alternatively, the practitioner can amplify the human subject's nucleic acids by in vitro cell culture and harvest of the subject's cultured cells, or by multiple sampling from the subject's tissues in vivo and pooling of multiple tissue samples from a subject. Nucleic acids thus "amplified," if they comprise a *PARP* gene or a part thereof, are "amplification products" for purposes of the present invention.

In a most preferred embodiment of the present method, PCR is used for amplifying the subject's nucleic acids, particularly from a *PARP* region. Briefly, a thermal resistant DNA polymerase (e.g., *Taq* polymerase), and *PARP*-specific primers are employed to amplify the genomic DNA to produce *PARP* gene-specific amplification products. If PCR is used, any combination of oligonucleotide primers that will amplify nucleic acid sequences of a *PARP* region, or parts thereof, can be employed. Useful primer sequences are readily available, for example, from the GenBank database. Most preferably, amplification of the subject's nucleic acids can be achieved using a set of oligonucleotide primers comprising a 5' (forward) primer sequence GAT TCC CCA TCT CTC TTT CTT T (SEQ. ID. NO.:1) or a 3' (reverse) primer sequence AAA TTG

TGG TAA TGA CTG CA (SEQ. ID. NO.:2), or a fragment of either of these at least 18 nucleotides long. But also preferred in practicing the inventive method is any other embodiment of the inventive primer set as described hereinbelow.

A primer set of the present invention, for detecting in a human subject a genetic susceptibility to SLE, functions to initiate nucleic acid synthesis in PCR from both the 5' and 3' ends of a nucleic acid template comprising a *PARP* region or part thereof. The primer set of the present invention comprises any two suitable oligonucleotide primers of the present invention as described above. In a most preferred embodiment, a primer set of the present invention comprises primers having (SEQ. ID. NOS.:1 and 2), or fragments thereof each at least 18 nucleotides long. But also preferred is a primer set having a forward primer sequence, of about 15 to about 30 nucleotides in length, corresponding to a *PARP*-specific sequence of (SEQ. ID. NO.:5) entirely 5' to nucleotide position 846 of the Ogura *et al.* *PARP* reference sequence (SEQ. ID. NO.:5; Biochem. Biophys. Res. Commun. 167(2):701-710 [1990]), and a reverse primer sequence, of about 15 to about 30 nucleotides in length, corresponding by complementarity to a *PARP*-specific sequence of (SEQ. ID. NO.:5) entirely 3' to nucleotide position 869 of (SEQ. ID. NO.:5), such that a detectable *PARP*-specific amplification product can be produced in the PCR reaction mixture when genomic DNA containing the *PARP* gene is present in the sample.

*PARP*-specific polynucleotides are determined by base sequence similarity or homology to (SEQ. ID. NO.:5). Base sequence homology is determined by conducting a base sequence similarity search of a genomics data base, such as the GenBank database of the National Center for Biotechnology Information (NCBI; [www.ncbi.nlm.nih.gov/BLAST/](http://www.ncbi.nlm.nih.gov/BLAST/)), using a computerized algorithm, such as PowerBLAST, QBLAST, PSI-BLAST, PHI-BLAST, gapped or ungapped BLAST, or the "Align" program through the Baylor College of Medicine server ([www.hgsc.bcm.tmc.edu/seq\\_data](http://www.hgsc.bcm.tmc.edu/seq_data)). (E.g., Altchul, S.F., *et al.*, *Gapped BLAST and PSI-BLAST: a new generation of protein database search programs*, Nucleic Acids Res. 25(17):3389-402 [1997]; Zhang, J., & Madden, T.L., *PowerBLAST: a new network BLAST application for interactive or automated sequence analysis and annotation*, Genome Res. 7(6):649-56 [1997]; Madden, T.L., *et al.*, *Applications of network BLAST server*, Methods Enzymol. 266:131-41 [1996]; Altschul, S.F., *et al.*, *Basic local alignment search tool*, J. Mol. Biol. 215(3):403-10

[1990]). Preferably, a *PARP*-specific polynucleotide sequence is at least 5 to about 30 contiguous nucleotides long, more preferably at least 6 to 15 contiguous nucleotides long, and most preferably at least 7 to 10 contiguous nucleotides long.

A genetic testing kit of the present invention, for detecting in a human subject a genetic susceptibility to SLE, is a ready assemblage of materials for facilitating amplifying nucleic acids from a human subject comprising *PARP* nucleotide sequences and/or analyzing or detecting *PARP*-derived amplification products. A genetic testing kit of the present invention comprises at least one oligonucleotide primer of the present invention and preferably comprises a primer set of the present invention, together with instructions for the practice of the methods of the present invention. The materials or components assembled in the present genetic testing kits can be provided to the practitioner stored in any convenient and suitable ways that preserve their operability and utility. For example the components can be in dissolved, dehydrated, or lyophilized form; they can be provided at room, refrigerated or frozen temperatures.

A most preferred embodiment of the genetic testing kit of the present invention comprises a primer set having (SEQ. ID. NOS.: 1 and 2), or fragments thereof each at least 18 nucleotides long, together with instructions for practicing the present method.

A preferred embodiment of the genetic testing kit of the present invention can incorporate an array of oligonucleotide primers specific for SNPs in human *PARP*, preassembled in a "DNA chip" (or "gene chip") configuration for facilitating the amplifying of nucleic acids and the analyzing or detecting of amplification products. (E.g., J.G.Hacia *et al.*, *Enhanced high density oligonucleotide array-based sequence analysis using modified nucleoside triphosphates*, Nucleic Acids Res. 26(2):4975-82 [1998]; R.W. Wallace, *DNA on a chip: serving up the genome for diagnostics and research*, Mol. Med. Today 3(9):384-89 [1997]; T. Pastinen *et al.*, *Minisequencing: a specific tool for DNA analysis and diagnostics on oligonucleotide arrays*, Genome Res. 7(6):606-14 [1997]; M.T. Cronin *et al.*, *Cystic fibrosis mutation detection by hybridization to light-generated DNA probe arrays*, Hum. Mutat. 7(3):244-55 [1996]; A.C. Pease *et al.*, *Light-generated oligonucleotide arrays for rapid DNA sequence analysis*, Proc. Natl. Acad. Sci. USA 91(11):5022-26 [1994]; E.M. Southern *et al.*, *Arrays of complementary oligonucleotides for analyzing the hybridisation behaviour of nucleic acids*, Nucleic Acids Res. 22(8):1368-73



[1994]).

In a preferred embodiment of the method of the present invention, nucleotide sequencing of *PARP* can be used to analyze the amplification products of the nucleic acids in a human subject's tissue sample to detect a variant allele in a *PARP* gene. A variant allele of *PARP* can be detected  
5 by any nucleotide sequencing means, for example conventional dideoxy sequencing or preferably by using a commercially available automated sequencer, then by comparing the subject's nucleotide sequences to other known human *PARP* sequences available in genomic sequence databases, such as GenBank.

In a most preferred embodiment of the present method that employs nucleotide sequencing,  
10 sequencing of *PARP* may be accomplished by using fluorescence-based single strand conformation polymorphism analysis (SSCP), a routine and reliable means of identifying point mutations, small insertions or deletions. (J.S. Ellison, *Fluorescence-based mutation detection. Single-strand conformation polymorphism analysis [F-SSCP]*, *Mol. Biotechnol.* 5(1):17-31 [1996]; H. Iwahana  
15 *et al.*, *Multiple fluorescence-based PCR-SSCP analysis using internal fluorescent labeling of PCR products*, *Biotechniques* 21(3):510-14, 516-19 [1996]; R. Makino *et al.*, *F-SSCP: fluorescence-based polymerase chain reaction-single-strand conformation polymorphism [PCR-SSCP]*, *PCR Methods Appl.* 2(1):10-13 [1992]). An automated system may be used, such as an Applied Biosystems DNA sequencer, equipped with GENESCAN 672, Genetyper, or another appropriate analytical software package.

20 Optionally, high throughput analysis is achieved by PCR multiplexing techniques well known in the art. (E.g., Z. Lin *et al.*, *Multiplex genotype determination at a large number of gene loci*, *Proc. Natl. Acad. Sci. USA* 93(6):2582-87 [1996]).

In another preferred embodiment of the present method, nucleotide sequencing is unnecessary for analyzing or detecting the amplification products. For example, heteroduplex  
25 analysis on high resolution gel matrices can be employed by the skilled practitioner to detect even single nucleotide polymorphisms. (M.T. Hauser *et al.*, *Generation of co-dominant PCR-based markers by duplex analysis on high resolution gels*, *Plant. J.* 16(1):117-25 [1998]). The PCR/OLA procedure can be used for analyzing or detecting amplification products to detect SNPs in *PARP*. (B.R. Glick and J. J. Pasternak, *Molecular Biotechnology: Principles and Applications*

of *Recombinant DNA*, ASM Press, Washington, D.C., pp. 197-200 [1994]). Conformation-sensitive gel electrophoresis of amplification products can also be employed as a means of analysis by the skilled artisan in practicing the methods of the present invention. (A. Markoff *et al.*, *Comparison of conformation-sensitive gel electrophoresis and single strand conformation polymorphism analysis for detection of mutations in the BRCA1 gene using optimized conformation analysis protocols*, Eur. J. Genet. 6(2):145-50 [1998]).

A skilled practitioner will know that electrophoresis for analyzing or detecting amplification products is done rapidly and with high sensitivity by using any of various methods of conventional slab or capillary electrophoresis, with which the practitioner can optionally choose to employ any facilitating means of nucleic acid fragment detection, including, but not limited to, the use of radionuclides, biotin, UV-absorbance or laser-induced fluorescence. (K. Keparnik *et al.*, *Fast detection of a (CA)18 microsatellite repeat in the Ig E receptor gene by capillary electrophoresis with laser-induced fluorescence detection*, Electrophoresis 19(2):249-55 [1998]; H. Inoue *et al.*, *Enhanced separation of DNA sequencing products by capillary electrophoresis using a stepwise gradient of electric field strength*, J. Chromatogr. A. 802(1):179-84 [1998]; N.J. Dovichi, *DNA sequencing by capillary electrophoresis*, Electrophoresis 18(12-13):2393-99 [1997]; H. Arakawa *et al.*, *Analysis of single-strand conformation polymorphisms by capillary electrophoresis with laser induced fluorescence detection*, J. Pharm. Biomed. Anal. 15(9-10):1537-44 [1997]; Y. Baba, *Analysis of disease-causing genes and DNA-based drugs by capillary electrophoresis. Towards DNA diagnosis and gene therapy for human diseases*, J. Chromatogr B. Biomed. Appl. 687(2):271-302 [1996]; K.C. Chan *et al.*, *High-speed electrophoretic separation of DNA fragments using a short capillary*, J. Chromatogr B. Biomed. Sci. Appl. 695(1):13-15 [1997]).

For example, in a most preferred embodiment using a primer set containing SEQ. ID. NOS.:1 and 2 to amplify the nucleic acids, the presence of an 85 bp fragment (the 85 bp allele) is associated with a diagnosis of SLE or an increased genetic susceptibility to developing SLE in subjects of diverse ethnic origins. (See Table 2). Another example is a 97 bp fragment, product of PCR amplification using the same primer set, which indicates the 97 bp allele and is associated with decreased genetic susceptibility to SLE. Consequently, the absence of a 97 bp fragment indicates an increased genetic predisposition to develop SLE.

Any of diverse fluorescent dyes are optionally used to label primers or amplification products for ease of analysis, including but not limited to, SYBR Green I, YO-PRO-1, thiazole orange, Hex (i.e., 6-carboxy-2',4',7',4,7-hexachlorofluoroscein), FAM (i.e., 6-carboxyfluorescein), or TET (i.e., 4,7,2',7'-tetrachloro-6-carboxyfluoroscein). (E.g., J. Skeidsvoll and P.M. Ueland, *Analysis of double-stranded DNA by capillary electrophoresis with laser-induced fluorescence detection using the monomeric dye SYBR green I*, Anal. Biochem. 231(20):359-65 [1995]; H. Iwahana *et al.*, *Multiple fluorescence-based PCR-SSCP analysis using internal fluorescent labeling of PCR products*, Biotechniques 21(30):510-14, 516-19 [1996]).

Analyzing or detecting the amplification products can also be done by means of restricting the amplification products with one or more restriction enzymes, followed by separation of the resulting fragments and analysis of fragment length or differential fragment migration in denaturing high-performance liquid chromatography (DHPLC) or gel electrophoresis, as above, including restriction-capillary electrophoresis. For example, this may be achieved by techniques known in the art, such as PCR-restriction fragment-SSCP, which can detect single base substitutions, deletions or insertions. (M. Tawata *et al.*, *A mass screening device of genome by polymerase chain reaction-restriction fragment-single strand conformation polymorphism analysis*, Genet. Anal. 12(3-4):125-27 [1996]; H.H. Lee *et al.*, *Mutational analysis by a combined application of the multiple restriction fragment-single strand conformation polymorphism and the direct linear amplification DNA sequencing protocols*, Anal. Biochem. 205(2):289-93 [1992]).

"Genetic predisposition" or "susceptibility" to SLE, or to develop SLE, means that a human subject having such a predisposition or susceptibility possesses an hereditary inclination or tendency to develop SLE. This does not mean that such a person will actually develop SLE at any time, merely that he or she has a greater likelihood of developing SLE compared to the general population of individuals that is not homozygous or heterozygous for a variant allele of a *PARP* gene or does not possess a *PARP* genotype or polymorphism associated with increased SLE risk.

The present invention provides a rapid and accurate genetic test for predicting susceptibility to SLE. It can be applied diagnostically in conjunction with other diagnostic tests in suspected cases of SLE to confirm a diagnosis of SLE. Previously available diagnostic tests for SLE are limited in their diagnostic effectiveness. Therefore, the methods, primers, primer sets, and genetic

testing kits of the present invention provide a useful genetic test for SLE that significantly enhances the existing diagnostic armamentarium for SLE.

In addition, it is contemplated that the genetic testing methods, oligonucleotide primers, primer sets, or genetic testing kits of the present invention can be used therapeutically to detect and treat SLE patients with specific variant alleles of *PARP*. These variant alleles can be targeted for gene therapy directed at repairing malfunctional *PARP* alleles or they may suggest specific chemotherapy directed to enhancing endogenous poly(ADP-ribosyl)transferase activity or may be directed to other metabolic targets in biochemical pathways mediated by poly(ADP-ribosyl)transferase activity.

The methods, primers, primer sets, and genetic testing kits of the present invention are superior to previously available diagnostic tools for SLE, because the present invention can be applied so that a person not presenting symptoms of SLE, but who is found to possess a genetic predisposition for developing SLE, can be treated prophylactically or can make lifestyle changes to minimize his or her exposure to environmental risk factors in order to prevent the development of SLE. A person seeking genetic testing for this purpose is likely to be one with a known family history of SLE, but the genetic testing methods of the present invention are equally applicable to healthy individuals without any known family history of SLE.

The prophylactic value of genetic testing for a genetic predisposition for developing SLE can be related to a variety of environmental and physiological risk factors. For example, exposure to sunlight is a risk factor for precipitating SLE. (I. Krause *et al.*, *Seasons of the year and activity of SLE and Behcet's disease*, Scand. J. Rheumatol. 26(6):435-391997]). The ultraviolet radiation in sunlight may cause damage to nuclear DNA in skin cells, resulting in apoptosis in keratinocytes, that may lead to SLE. (L. Casciola-Rosen and A. Rosen, *Ultraviolet light-induced keratinocyte apoptosis: a potential mechanism for the induction of skin lesions and autoantibody production in LE*, Lupus 6(2):175-80 [1997]; F. Nyberg *et al.*, *Early detection of epidermal dust-like particles in experimentally UV-induced lesions in patients with photosensitivity and lupus erythematosus*, Acta Derm. Venereol. 78(3):177-79 [1998]; T. Hasan *et al.*, *Photosensitivity in lupus erythematosus, UV photoprovocation results compared with history of photosensitivity and clinical findings*, Br. J. Dermatol. 136(5):699-705 [1997]; L.C. Korb and J.M. Ahearn, *C1q binds*

*directly and specifically to surface blebs of apoptotic human keratinocytes: complement deficiency and systemic lupus erythematosus revisited*, J. Immunol. 158(10):4525-28 [1997]).

Persons who are found, through the use of methods, primers, primer sets, or genetic testing kits of the present invention, to have a predisposition for developing SLE can be advised of their particular need to minimize or avoid exposure to sunlight in order to lower SLE risk.

Also, estrogen exposure, another environmental risk factor for SLE (B.L. Kotzin [1996]), can be minimized for menopausal women who are found to have a genetic predisposition to develop SLE. Women with naturally or surgically induced menopause commonly receive estrogen replacement therapy to relieve the symptoms of ovarian hormone deficiency, including vasomotor instability and osteoporosis. Menopausal women with a known genetic predisposition to develop SLE, as detected by the methods of the present invention, can avoid standard estrogen replacement therapy, perhaps in favor of alternative hormone replacement therapies, for example, various hormone replacement combinations or alternative androgen replacement therapies known in the art. Thus, information, provided by practicing the present invention, that a genetic predisposition to develop SLE exists in a particular woman, can help that women minimize or avoid estrogen exposure in order to lower SLE risk.

At least in some Asian populations, a history of menstrual irregularity is reported to be a risk factor for SLE. (Y. Minami *et al.*, *Female systemic lupus erythematosus in Miyagi Prefecture, Japan: a case-control study of dietary and reproductive factors*, Tohoku J. Exp. Med. 169(3):245-52 [1993]). By using the methods of the present invention to find a genetic predisposition for developing SLE, a more informed decision can be made whether to prescribe a premenopausal woman with both this risk factor, and a predisposition for SLE, appropriate hormonal therapy to regularize her period.

Similarly, because frequent intake of fatty meat, such as beef or pork, is associated with increased SLE risk, at least in some populations (Y. Minami *et al.* [1993]), knowledge that a particular woman has a genetic predisposition to develop SLE, can inform the practitioner to prescribe a preventative change in diet for that woman.

Also, results obtained through practicing the methods of the present invention can be used in genetic counseling to provide useful information to persons considering their reproductive

options. A couple in which one or both of the partners is found to be homozygous or heterozygous for a variant allele of *PARP*, such as, but not limited to, the 85 bp allele of the *PARP* promoter region, can consider this fact in making informed reproductive choices with respect to SLE risk affecting their future offspring. For example, as determined in the detailed examples below, the risk of developing SLE with one 85 bp allele compared to having none is 2.3 while it is 4.0 with two 85 bp alleles. Such information can be factored by those skilled in the art of genetic counseling, when calculating the SLE risk for offspring.

The foregoing applications for the methods, oligonucleotide primers, primer sets, and genetic testing kits of the present invention are illustrative and by no means exhaustive. The invention will now be described in greater detail by reference to the following non-limiting examples.

## EXAMPLES

### Susceptibility to SLE is linked to *PARP* alleles.

Tsao *et al.* [1997] had analyzed, for linkage to SLE, 7 microsatellite markers present on the human chromosomal region (1q31-q42) corresponding to the telomeric end of mouse chromosome 1, the latter being the region where contributions to specific manifestations of murine lupus, including glomerulonephritis and IgG anti-chromatin. A linkage analysis of 51 sibpairs (from three ethnic groups) fulfilling 1982 American College of Rheumatology criteria for SLE showed an increased sharing of markers and haplotypes when compared to the expected random distribution ( $p < 0.01$  by a goodness-of-fit  $\chi^2$ ). Separate analyses of these 7 markers, comparing the mean marker sharing of each to the expected value of 0.50, resulted in no evidence for linkage with the two markers present on 1q31-q32 while the five markers located at 1q41-q42 showed significantly increased marker sharing ( $p = 0.0005 - 0.08$ ). Also, the quantitative trait, IgG anti-chromatin, showed linkage to one of the five markers ( $p = 0.04$ ) and the combined marker haplotype ( $p < 0.06$ ), evidence that the phenotypic consequences of this susceptibility locus are conserved between mice and humans.

Within the 1q41-q42 human chromosomal region, there are no recognized immunologic candidate genes to account for these results. Therefore, the 1q41-q42 region was examined for SLE susceptibility gene(s), as detailed in the following examples.

### **Example 1. Human Subjects:**

This study was approved by the Human Subject protection Committee of the University of California, Los Angeles (UCLA). These families were recruited at UCLA, and at other collaborating sites including the University of Texas, Houston (FCA; the Zentralkrankenhaus Reinkenheide in Bremerhaven, Germany (KH), and Ottawa General Hospital in Canada (RG). Simplex families were mainly recruited through UCLA rheumatologists to include SLE patients, their parents, and other sibs if available. Medical records for SLE patients were either reviewed by UCLA rheumatologists or were provided by the patients' rheumatologists in the form of completed checklists, and the checklist information was then entered into the UCLA database. This database was used for classification of patients as SLE (requiring at least 4 of the 11 American College of Rheumatology Classification criteria). (E.M. Tan *et al.*, *Special article. The 1982 revised criteria for the classification of SLE*, *Arthritis Rheum.* 25:1271-7 [1982]). Siblings of SLE patients who had no positive responses in the Connective Tissue Screening Questionnaire (W.E. Karlson *et al.*, *A connective tissue disease screening questionnaire for population studies*, *Ann. Epidemiol.* 5:297-302 [1995]) were classified as unaffected sibs. Multiplex families were recruited by ascertaining nuclear families for two or more SLE patients which were extended to include parents and other siblings.

The subject sample included 78 SLE-affected sibpairs from Caucasian, Asian, and African-American families. All participating members (77 parents, 134 affected and 89 unaffected siblings) were genotyped using 14 microsatellite markers within a 30 cM region (Figure 1) containing the 15 cM region of human chromosome 1q41-q42.

### **Example 2. Genotyping:**

Human genomic DNA was isolated from peripheral blood cells. Microsatellite markers at or near the specific candidate chromosomal region were selected based on the composite map accessible at [cedar.genetics.soton.ac.uk/pub/chrom1/gmap](http://cedar.genetics.soton.ac.uk/pub/chrom1/gmap). The primers for these marker were purchased from Research Genetics, Inc. (Huntsville, AL). Microsatellite genotyping was determined by scoring the size of PCR products. PCR was performed in a 96-well plate using a thermocycler which was programmed for 95°C for 1 min, 58°C for 30 s, and 72°C for 1 min, with

25 cycles. The labeled PCR products were denatured and separated on a sequencing gel of 5% polyacrylamide and 6 M urea.

To determine genotypes of *PARP*, we used the 5' oligonucleotide primer sequence 5'-GAT TCC CCA TCT CTC TTT CTT T-3' (SEQ. ID. NO.:1) tagged with the fluorescent dye 6FAM and the 3' oligonucleotide primer sequence 5'-AAA TTG TGG TAA TGA CTG CA-3' (SEQ. ID. NO.:2). The PCR condition for this polymorphism was 30 cycles of 93°C for 1 min, 56°C for 30 s, and 72°C for 1 min plus a final extension of 72°C for 5 min. For the intronic dinucleotide repeat of *HLXI*, the 5' oligonucleotide primer sequence was 5'-TTC ACA CAA GTT CCA GCT TCC CTT-3' (SEQ. ID. NO.:3) tagged with the fluorescent dye TET, and the 3' oligonucleotide primer sequence was 5'-TGC TGT CTC TGT TTC TTT CTG ACC-3' (SEQ. ID. NO.:4). The *HLXI* PCR condition was 10 cycles of 94°C for 15 s, 55°C for 15 s, and 72°C for 30 s, then followed by 20 cycles of 89°C for 15 s, 55°C for 15 s and 72°C for 30 s with a final extension at 72°C for 10 min. For these two candidate genes, PCR mixture contained 40 ng genomic DNA, 20 mM Tris-HCL pH8.0, 2 mM MgCl<sub>2</sub>, 10 mM KCl, 6 mM (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 0.1% Triton X-100, 10 µg/ml BSA, 0.5 Units of native *Pfu* DNA polymerase (Stratagene, San Diego, CA), 200 µM dNTP, and 0.2 µM primers in 5 µl reaction. Aliquots of fluorescent PCR products were electrophoresed using a 377 Prism ABI sequencer (Applied Biosystems, Foster City, CA) and analyzed by GeneScan and Genotyper programs.

### **Example 3. Data Analysis:**

Multipoint linkage analysis of 14 microsatellite markers in the chromosome 1q31-42 region and the SLE phenotype (affected or unaffected) was conducted on nuclear families using the MAPMAKERS/SIBS program (L. Kruglyak and E.S. Lander, *Complete multipoint sib-pair analysis of qualitative and quantitative traits*, Am. J. Hum. Genet. 57:439-54 [1995]) on a SUN ultra 60 workstation with a UNIX operating system. The program simultaneously uses information at multiple markers in a region to estimate the expected proportions of sib pairs selected for a disorder sharing 0, 1 and 2 alleles identical-by-descent for points along the chromosomal region. A lod score is generated by taking the log of the likelihood of the estimated allele sharing proportion compared to the theoretical likelihood of no linkage to the region. All markers except



*HLXI* were used in this analysis. The heuristic guideline of one lod below the peak value was used to identify a confidence interval of 5 cM for the location of the SLE susceptibility gene.

The transmission disequilibrium test (TDT; R.S. Spielman *et al.*, *Transmission test for linkage disequilibrium of the insulin gene region and insulin-dependent diabetes mellitus [IDDM]*, Am. J. Hum. Genet. 52:506-16 [1993]) can detect an allelic association with disease in the presence of linkage with the disease. The method involves assessing the transmission of alleles from heterozygous parents to their affected offspring, and testing whether there is evidence that the probability of transmitting the alleles at a marker is different than 0.5 for each allele. The Extended TDT (ETDT; P.C. Sham and D. Curtis, *An extended transmission/disequilibrium test [TDT] for multi-allele marker loci*, Ann. Hum. Genet. 59:323-336 [1995]), which uses a logistic regression approach to test all alleles at a marker for deviation from a 0.5 transmission probability simultaneously has been used (software provided by the program authors). When a deviation is detected in the overall test it examines each allele separately for a deviation from a 0.5 probability of transmission. The eldest affected individual has been used from each of the nuclear families, as due to linkage with the region, other affected sibs from the same families are not independent of those in this sample.

The relative risk for the 85 bp allele identified by the ETDT at the *PARP* was assessed using a method described by Weinberg *et al.* (C.R. Weinberg *et al.*, *A log-linear approach to case-parent-triad data: assessing effects of disease genes that act either directly or through maternal effects and that may be subject to parental imprinting*, Am. J. Hum. Genet. 62:969-78 [1998]). Poisson regression analysis was applied to data on the numbers of parent-child triads observed among the eldest affected offspring with at least one parent heterozygous for the 85 bp allele. The risk of developing SLE with one or two 85 bp alleles were estimated by using the GENMOD procedure of the statistical Analysis Stems (SAS) package of computer programs.

#### **Example 4. Results:**

As shown in Figure 1, multi-point linkage analysis on the current sample using the model-free MAPMAKER/SIBS program identified a peak with a lod score of 3.3 and narrowed the region of interest to approximately 5 cM between microsatellite markers D1S2860 and D1S213.

There were 12 *PARP* alleles detected in our cohort of 124 multiplex and simplex families. When data from the four most frequent alleles of *PARP* were analyzed by the multiallelic TDT, the overall skewing of transmission to affected offspring was evident in the combined sample containing all ethnic groups ( $p = 0.00008$ ) as well as individually in the Caucasian sample ( $p = 0.003$ ) (Table 1). Other ethnic groups were too small in number for valid analysis.

**Table 1. Skewed transmission of all analyzed *PARP* alleles.**

Groups	p-value	
	affected offspring	unaffected offspring
All ethnic groups combined	0.00008	0.02
Caucasians	0.003	0.08

Data presented are derived from the four most frequent *PARP* alleles transmitted and not transmitted from heterozygous parents to offspring in 124 families containing 75 Caucasian, 25 Hispanic, 17 Asian, and 7 African-American. P-value reflects the level of overall departure from the expected random distribution of all ethnic groups combined, or the Caucasian families only, as evaluated by a likelihood ratio test.

Results of a TDT analysis of the four most frequent alleles showed that the 85 bp allele of *PARP* was preferentially transmitted to affected offspring (transmission : non-transmission = 65 : 29,  $p = 0.0002$ ) and preferentially not transmitted to unaffected offspring (transmission : non-transmission = 25 : 50,  $p = 0.004$ ) (Table 2). The risk of developing SLE with one 85 bp allele compared to having none is 2.3. while it is 4.0 with two 85 bp alleles. The 85 bp allele or polymorphism is a CA repeat located about 907 bp upstream of the transcription start site. (See T. Ogura *et al.* [1990]). Results of a TDT analysis of the four most frequent alleles showed that the 85 bp allele of *PARP* was preferentially transmitted to affected offspring (transmission : non-transmission = 65 : 29,  $p = 0.0002$ ) and preferentially not transmitted to unaffected offspring (transmission : non-transmission = 25 : 50,  $p = 0.004$ ) (Table 2). The risk of developing SLE with

one 85 bp allele compared to having none is 2.3, while it is 4.0 with two 85 bp alleles. The 85 bp allele or polymorphism is characterized by a twelve-fold CA dinucleotide repeat sequence located upstream of the transcription start site, which start site (designated position +1 in Ogura et al. [1990], italic “t”) is at position 1777 of the Ogura reference sequence (SEQ. ID. NO.:5), as described by Ogura *et al.* (Biochem. Biophys. Res. Commun. 167(2):701-710 [1990]). Thus, when the 85 bp allele is present, a 24-bp nucleotide sequence 5'-cacacacacacacacacacaca-3' (SEQ. ID. NO.:6, bold) is detectable in the *PARP* genomic sequence and in the amplification products, which CA dinucleotide repeat sequence corresponds to nucleotide positions 846 through 869 of the Ogura reference sequence (SEQ. ID. NO.:5), which is consecutive nucleotide positions -908 through -931 in relation to the transcription start site (+1), as described in Ogura *et al.* The Ogura *et al.* reference sequence (SEQ. ID. NO.:5; GenBank Accession X16674.1; GI:510112) is the following:

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1 tttagggatg atatagttgt caaccagag atggcatgat catgccttt gacttggtca
61 ttctctaagt aaaacttta ttgttccat catattttcc acttattctg ttacettca
121 aaatatcttt tttttttt ttgagacag ggtcacactg tcaccaggc tagagtccag
181 tggcactatc atggctcacc acagcctcaa ccttcagggc tcagggtgatc ctcccacttc
241 agcctccga gtagatggga ctacaggcac ctgccaccac cccagctaa ttttgtaga
301 gacaaggttt tgccatgttg tccaggctgg tcttgaactc ctgggctcaa gggatccggc
361 cacctcagcc tcccaaagtg ctaggattat aggcatgagc cactgtgccc agcctacctt
421 caacgtatct aactggttac taacttttag gattcggcct atgtctaca accttcttgc
481 ttactcaaca tcctgtctc ttaagccact agcttcttct ctatgggtaa cactttttat
541 gagttttatt catctgctta ttttcttat cctctatacc agaattgaat atttcaaat
601 aaagcacact catgttaca tctttgaaat ggaaaaaaaa aatgcatagg attagaaaag
661 aaaccaattt taataaacta tattttgaag tatagttcta tattaaacaa caagatctag
721 gccagggtgca gtggctcatg cctgtaatcc cagcaatttg ggaagtcgag gtgggaggat
781 tgcttgaggc caggggttca agaccagcct gggcaacatg gagagattcc ccatctcttt
841 ctttacacac acacacacac acacacacaa aatatctgat agcaacaggt gcagtcatta
901 ccacaatttc gagtagtgat gagcttaata atatttcgag ttatcaccaa caactgtaaa

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a primer pair or set to amplify the genomic DNA. However, other suitable *PARP*-specific primer sequences can be used to detect the presence or absence of (SEQ. ID. NO.:6), i.e., the 85-bp allele, or (SEQ. ID. NO.:7), i.e., the 97-bp allele, as long as the forward primer sequence selected corresponds to a sequence entirely 5' to nucleotide position 846 of (SEQ. ID. NO.:5), and the reverse primer sequence selected is complementary to a sequence entirely 3' to nucleotide position 869 of (SEQ. ID. NO.:5).

**Table 2. Preferential transmission of *PARP* allele 85 to affected but not to unaffected offspring.**

Alleles (bp)	Affected Offspring <sup>a</sup>				Unaffected Offspring <sup>b</sup>			
	t	nt	%t	p-value	t	nt	%t	p-value
85	65	29	69	0.0002	25	50	33	0.004
93	23	33	41	0.18	21	20	51	0.87
95	12	15	44	0.56	16	3	84	0.003
97	17	40	30	0.002	30	19	61	0.11

<sup>a,b</sup>The oldest affected or unaffected offspring in each family was used for this analysis. Data presented are numbers of times that *PARP* alleles were transmitted (t) and not transmitted (nt) from heterozygous parents. Data are shown as the percentage transmission of each allele (%t). Each p-value is the level of significance based on a test of the expected random (50%) allele transmission and was evaluated using a chi-squared statistic ( $\chi^2 = [t-nt]^2 / (t+nt)$ ) with 1 degree of freedom).

In contrast, the 97 bp allele of *PARP* might be protective, as it was preferentially not transmitted to affected offspring (transmission : non-transmission = 17: 40, p = 0.002)(Table 2).

Similar TDT analyses of *HLXI* alleles, using an intronic dinucleotide repeat within this gene, yielded no evidence for association with SLE. Transmission data of 7 *HLXI* alleles to affected offspring from 129 heterozygous Caucasian parents are summarized in Table 3; no significant difference from the expected random distribution was found (the chi-squared for genotype-wise TDT = 11.35, p = 0.88).

**Table 3. HLX1 alleles are not associated with susceptibility to SLE.**

	Alleles (bp)	Affected Offspring		
		t	nt	%t
5	168	9	8	53%
	166	10	9	53%
	164	8	11	42%
	162	17	18	49%
	160	30	30	50%
10	158	39	32	55%
	152	16	21	43%

The abbreviations (t, nt, %t, and p-value) are the same as those described in Table 2. The analyzed Caucasian families contained 129 heterozygous parents. The overall transmission of 7 tested alleles was not different from the expected random distribution with a p-value of 0.88.

15 The contrasting TDT results of these two candidate genes, *PARP* and *HLX1* demonstrate that *PARP* is a susceptibility gene for SLE within the linked 1q41-q42 region.

The foregoing examples being illustrative but not an exhaustive description of the embodiments of the present invention, the following claims are presented.